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Contents

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Introduction

Red clover (*Trifolium pratense* L.) has not been grown extensively in Louisiana, but has had limited use, primarily as a pasture and hay crop. Those who have grown it have been generally pleased with its performance. Red clover has been grown in areas north of Tennessee and Virginia for many years because most varieties and strains developed in the past have been acclimated to such a region. Field trials have shown that such varieties and strains have not generally been adapted to environmental conditions farther south. This crop is best adapted to the heavier soil types. Fertility requirements of red clover are similar to those of white clover, and perhaps not so exacting as those of alfalfa. The nutritive value of red clover approaches that of alfalfa. Objections have been raised by some that red clover hay does not have a green color. This loss of color, thought to be due to an enzyme present in the leaves, does not affect the palatability or nutritive value of the hay.

In past years, red clover varieties have been developed by the influence of local conditions rather than by any formal breeding practices (*12*)[2]. There are varieties and strains that developed in the southern region by becoming acclimated to the area. These were found to be high producers of forage, but were lacking in resistance to foliage diseases, especially powdery mildew (*Erysiphe polygoni* D.C.). This disease, while not a serious hazard to the production of this crop for pasturing animals, is considered highly objectionable when clover is cut for hay. This fungus is present on mature leaflets, and under grazing a large portion of the leaves are consumed prior to maturity.

A breeding program was initiated with red clover in 1945 to develop improved varieties adapted to Louisiana. The principal objectives were to develop varieties that would produce high forage yields and be resistant to foliage diseases. Adapted varieties of this crop could add substantially to the quality of the forage produced in the state and to the economical production of livestock.

Most varieties of red clover observed in space plantings in nurseries were found to be variable for powdery mildew infection. This afforded an opportunity to develop disease-resistant strains. It was realized that such strains should be developed from acclimated stock to be of greater value for use in Louisiana. Red clover is classed as a perennial by botanists

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[2]Italic numbers in parentheses refer to Literature Cited, Page 16.

and behaves as such in the Midwest and in northern states. In Louisiana, in most years, it may be considered an annual. Consequently, seed-increase fields have been forced to reseed naturally, thereby developing the hard-seed characteristic within the variety Tensas.

Review of Literature

In the wild state, red clover ranges over most of Europe and far into Siberia (*12*). Pieters and Hollowell (*12*) said "the crop was known to be generally cultivated in the Netherlands 370 years ago and possibly there may have been an older culture of clover." The wild red clover is an extremely variable species. There are early, late, smooth, hairy, prostrate, erect, and semi-erect forms (*12*).

The many varieties that developed prior to about 1937, according to Pieters and Hollowell (*12*), were developed by action of local conditions rather than by conscious selection and testing. Nothing is definitely known, they stated, regarding the origin of the red clover now common to North America, with its hirsute or hairy leaves and stems.

Many varieties were known to be in existence prior to about 1940. These arose from strains that developed by natural means. Farmers usually recognized these as superior and kept them pure by natural isolation. Among such varieties were Nolin's in Louisiana and Port Gibson in Mississippi (*11*). Chesapeake red clover was found growing in an area of Maryland, and the seed found their way into market channels (*4*). Among the varieties developed by a formal breeding program were Tensas (*11*), Kenland, and Kenstar (*14*).

The genetics of red clover was reviewed by Attwood (*3*). He stated that the chromosome number of red clover was N = 14. The relationship of the fertility factor was of the diploid personate type of multiple oppositional alleles. The number of factors for sterility was considered to be extensive (*3*).

The factor S (for sterility) was shown by Williams (*21*) to be located on linkage group 2, where it was linked with the factor C/c for flower color. The mean cross-over value for S and C was found to be about 35 (*21*). Seven simple recessive factors governing the production of yellow chlorophyll-deficient seedlings were reported by Williams (*19*). These were reported as being different from each other and from all of the eight white seedling factors also reported by Williams (*18*). Williams (*20*), reporting on the genetics of red clover and its bearing on practical breeding, referred to the extensive series of factors for sterility. Twenty plants, which were studied for sterility constitution, were found to carry 34 different sterility alleles. He further stated that, from the standpoint of plant improvement, self-fertility was undesirable. Inbreeding invariably resulted in a marked and progressive loss of vigor.

Rinke and Johnson (*13*) reported a self-fertile clone of red clover. It

continued to be highly self-fertile in 10 subsequent generations. A method was outlined for the use of the self-fertile clone in a breeding program.

Williams and Williams (*17*) reported on the employment of the pseudo-self-fertility factor for producing selfed seed from the self-incompatible clones. Four homozygous genotypes were produced by the utilization of this factor present in certain lines. Leffel (*8*) reported on the use of the pseudo-self-fertility factor in the production of selfed seed in clones known to be self-incompatible. He stated that the degree of pseudo-self-compatibility (PSC) varied at different dates of determination, but clonal differences for this factor were generally consistent. Relatively high temperatures (90° F), he said, increased pseudo-self-compatibility of the four selected clones. Leffel and Muntjan (*7*) subjected clones of randomly selected red clover plants to various treatments suggested in the literature as affecting PSC. They reported that PSC was a definite clonal characteristic. Virus diseases, gibberellic acid, and wilting did not alter its expression.

The effects of maternal line selection for persistence were reported by Mokhtarzadeh, et al. (*10*). Anderson, et al., (*1*) discussed combining ability of open-pollinated clones of red clover. Anderson and Kirthavip (*2*), reporting on the development and performance of hybrid red clover, said most hybrids were not superior to the best check varieties. Taylor, et al., (*15*) discussed the results of inbreeding and heterosis. They failed to report specific gains from hybrids between inbred parents.

Nitrogen fixation by red clover was reported by Lyon and Bizzel (*9*). They included red clover in the earliest report on the benefits of growing legumes with oats. Other legumes included were alfalfa and winter peas. It was found that the protein content of the forage was increased when oats and timothy were grown with any of these legumes. Harlan (*6*) reported red clover grown in Finland produced a large amount of nitrogen.

Powdery mildew was described by Dickson (*5*) as an obligate parasite persisting on the foliage of red clover in moderate climates and apparently spreading rapidly late in the summer. The control, stated Dickson, was chiefly through use of resistant varieties.

Materials and Methods

Breeding Methods

The objective in breeding red clover was to develop superior types, strains, or varieties suited for growing in Louisiana and the South. At the time this research was initiated there were acclimated varieties growing within the state. Although they were productive, they lacked resistance to certain diseases prevalent in the area, especially powdery mildew.

During the summer of 1945, seed were procured from each of the acclimated strains, as well as from other red clover varieties grown in Louisiana. These were established in a space-planted field nursery by germinating the seed in the greenhouse, transferring the seedlings to

6-ounce paper cups filled with soil, and finally transplanting them to the field during April. About 2,500 seedlings were established. Selections were made from the plants that had survived the summer in thrifty condition and were relatively free of disease during late summer. Seed from these selected plants were planted in progeny row tests the following fall. Seed from the better progenies were carried forward to new strain experiments. Finally, seed from five of the most outstanding new strains were planted in adjacent rows in an isolated area and allowed to interpollinate. The seed from these strains were mixed and designated as Louisiana Strain 1.

Seed of Strain 1 were planted in a seed increase block and grown for a number of seasons. Seed were harvested each year and used for planting in yield trials and for conducting demonstrations. The clover was forced to reseed on the land each year.

This strain of red clover was found to excel in forage production, and it had less powdery mildew than the other varieties tested, yet during most seasons it had more powdery mildew infection than was desirable for good quality hay.

After the test period, Louisiana Strain 1 was given the variety name "Tensas." The lines from which it was formed were derived from seed that came from a farm in Tensas Parish.

Breeding for Resistance to Powdery Mildew

The program for developing strains of Tensas with resistance to powdery mildew was begun in 1958. The screening was started in the greenhouse, but it was found that even in the presence of mature plants that were infected with the disease, no discernible infection was visible on the seedlings. After two or three trials it was concluded that the greenhouse facilities were not suited for the work. It was thought that the temperature became too high in the greenhouse early in the season. Consequently, it was necessary to use field nurseries. A planting of several hundred plants, spaced three and one-half feet apart, was made in the fall. These were thinned to one plant per hill the following spring. When the plants had matured seed, individuals were selected that were free of the disease.

Progeny rows were established in a spaced nursery with seed from the selected plants. The seed were planted in hills spaced three and one-half feet apart in rows of the same width. Plants were thinned to one plant per hill the following spring. When the blossoming had begun, each plant was marked with a dated stake. After the beginning of the season for blooming, the nursery was observed daily until all of the plants had bloomed.

After the blossoming stage and when plants were near the mature stage, each plant was scored for powdery mildew infection (score of 0 to 10 for mildew-free to full cover of the leaves with the disease), and each was scored for size (0 to 15 for small to large). Mildew-infected plants were

removed from the nursery and destroyed. Mildew-free plants were cut back and new growth was allowed to arise from the crowns. The seed that were born on the regrowth from disease-free plants were harvested. The basis for making selections was vigor of the regrowths and the amount of seed produced. These progeny tests were repeated each year until the susceptible segregates were reduced to a minimum. The time was prolonged as a result of light infection some seasons and no infection at all during certain years.

In another area of the experimental field, isolated from the progeny nursery, similar experiments were conducted with certain varieties, including those known to be resistant to the disease and others known to be susceptible. The results from these tests were used as an indication of the prevalence of the disease spores. Forage yield trials were also conducted each year, and checks were made on the yields of entries formed from massed seed from the selected plants.

Experiments also were conducted to compare the forage production of red clover and white clover. Plots were seeded in the fall and forage was harvested the following April, May, and June. New plantings were made each year for these comparisons.

Figure 1.—Spaced nursery with mature plants.

Observations were made of the seed setting of varieties as well as of seed production and seed harvesting of the improved strains.

Results

The results from recurrent selection for powdery mildew resistance are shown in Table 1 for the years 1963 through 1972. These data were taken from progenies beginning the fourth generation after the program was started. The data show that in 1963 only four progenies out of 70 had no plants showing powdery mildew infection at full bloom. The mean score value for the entire nursery was 0.67. In 1964 there were 32 progenies that had no plants showing the disease at the stage of growth in which they were scored. The average score for all progenies was 0.30. In 1965, on the other hand, progenies of disease-free selections made from among the plants of the nursery in 1964, produced no progenies that were scored as being free of mildew. In fact, the infection was excessively high. Certain of the progenies were scored to be fully covered with mildew. The average for all progenies was 3.81. The difference in infection for the two seasons from practically the same genotypes was likely due to weather conditions or a different race of the disease organism.

The score data for the progenies the following year, 1966, showed an improvement but were still higher than for the other seasons during which the experiments were conducted. In 1969 there was very little infection from the disease.

Data in 1970 showed that of 100 progenies, not one was found to be infected. In 1971 only six of the total of 60 progenies were free of the disease. Infection was light, but there was some fungus present on many of the plants. Infection was also light in 1972; there were 16 progenies in

Table 1.—Distribution of progenies of red clover by classes of infection with powdery mildew

Year	Infection classes*											Mean
	0	0.5	1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	
	Number of progenies in each infection class											
1963	4	55	10	0	0	1	0	0	0	0	0	0.67
1964	32	48	0	0	0	0	0	0	0	0	0	0.30
1965	0	1	8	16	12	12	7	3	1	2	1	3.81
1966	7	36	15	6	3	1	2	0	0	0	0	1.16
1967	21	36	6	3	0	0	0	0	0	0	0	0.23
1969	65	1	0	0	0	0	0	0	0	0	0	0.01
1970	100	0	0	0	0	0	0	0	0	0	0	0.00
1971	6	41	12	1	0	1	0	0	0	0	0	0.71
1972	16	43	1	0	0	0	0	0	0	0	0	0.38

*Score values: 0 = no powdery mildew infection; 10 = full cover of leaflets with mildew. Scores are based on class centers.

which none of the plants was found to be infected at the full bloom stage. For those plants infected with the fungus, the scores amounted to less than 1.0. This should be considered as only a trace. The average score for 60 progenies in 1972 was 0.38. Score values were obtained from the average score for the plants in each progeny row at approximately full bloom. The average for the nursery included the mean infection score from all of the rows.

Tests using representatives of commercial varieties were conducted each year. Data from these experiments were used as an indication of the presence of the disease each season. Seed of these varieties were planted in spaced hills and managed in the same way as that of the progeny nursery. The tests were replicated and isolated from the progeny nursery.

Results shown in Table 2 indicate considerable variation from year to year in score values for disease infection. Inasmuch as infection was exceedingly light on the varieties in some years and heavy in others, it was assumed that the presence of an epidemic from year to year was uncertain and probably associated with fluctuations in weather conditions.

Powdery mildew is an obligate parasite (*5*), as the disease persists only in the presence of the host plants. Since the acreage of red clover is very small in Louisiana, the probability of obtaining an epidemic year after year is lessened in natural field plantings. Absence of mildew in 1964 and the presence of it to a considerable extent in 1965 might be attributed to a change in weather conditions. Mildew was present on the varieties in 1967 and 1968, but the varieties were less affected in 1969 and 1970.

Powdery mildew infection has not been a factor that would exclude the growth of red clover in south Louisiana during certain seasons. However, during years when infection prevails, plots planted to susceptible varieties have been almost entirely covered by the disease and the quality of forage has been reduced.

Table 2.—Varieties and strains of red clover compared for infection from powdery mildew, 1965-70

Variety or strain	1964	1965	1967	1968	1969	1970
	Powdery mildew infection score*					
Orbit	0	1.21	0.10	0.70	0.12	0
Tensas	0	2.60	0.42	0.78	0.12	0
Tensas, foundation seed	0	3.92	1.10	1.50	0.13	0
Tensas, parent	0	6.59	4.80	4.60	0.17	0.05
Kenland	0	—	—	—	0.83	0.28
Nolin's	0	8.57	—	5.60	0.09	0.06
LSD (5%)	—	0.97	1.35	1.51	—	—

*0 = no infection; 10 = fully covered with mildew.

Table 3.—Red clover strains developed from Tensas compared with the parent variety for powdery mildew infection, length of growing season, and dry weight of plants

Strains	Powdery mildew infection score*	Length of growing season, days	Dry weight of plants, pounds
Tensas, parent	4.80	162	0.61
Tensas, foundation seed	1.10	171	0.86
Tensas, breeder seed	1.10	172	1.30
Orbit	0.10	187	0.75
Massed seed of selected plants in 1965	0.17	173	1.00
Massed seed of selected plants in 1966	0.42	176	0.94
LSD (5%)	1.35	3.0	0.19

*0 = no infection; 10 = fully covered with mildew.

Comparison of New Strains with the Parent Variety and Orbit for Powdery Mildew Infection

During the 1967 season an experiment was conducted at Baton Rouge to compare new strains formed by the massing of the seed of a portion of the plant selections for 1965 and 1966. This experiment was conducted in a manner similar to that used with the progeny rows. It was,

Figure 2.—Single plant infected with powdery mildew.

however, planted in an area isolated from the progeny nursery.

The results from these experiments are shown in Table 3. Powdery mildew infection on the parent strain was moderate. Foundation seed produced plants with significantly less infection than the parent strain. Breeder seed produced plants that had the same score value as the foundation class. Orbit, which has more resistance to the disease than any of the other varieties and strains tested, had the lowest score. But the difference between Orbit and the entries formed by massing selected seed of mildew-free plants from the progenies selected from Tensas was not significant. The plants from the massed seed of the selected plants from progenies from 1965 and 1966 had lower scores for powdery mildew infection than plants from breeder or foundation seed, but they were not significantly lower at the 5 percent level of probability than those of Orbit. The fact that the scores for mildew were less from the new strains than from the plants from breeder seed or foundation seed indicated some progress. Specific studies were not made of the inheritance of resistance/susceptibility to powdery mildew. Observation led to the assumption that factors for resistance were dominant, but no further conclusions could be drawn.

Length of growing season was found to differ among strains and varieties. Strains developed from Tensas were later in maturing than the parent. Orbit was more than 3 weeks later in maturing than the parent, Tensas. The

Figure 3.—Single plant free of powdery mildew.

other entries, which were derived from the parent, were 10 to 15 days later than the parent strain. It may be thought that the stage of maturity affected the scoring. However, scoring was done for each entry when the plants had passed the full bloom stage so that they were at practically the same stage of maturity.

The average weight of plants produced by the different strains and varieties differed significantly. However, this has varied from season to season. In most years, the late plants produced more dry matter than those that matured early. It was not necessarily true, however, that strains which produced the large plants when space-planted gave high yields when sown in solid stands.

The new strains, which were evaluated for forage yields, mildew resistance, blooming date, and other characteristics, were transitional. That is, they were formed from a composite of plants selected from the progeny nurseries. This practice was repeated every second or third year during the period this work was underway.

Forage Yields of Varieties and New Strains

Forage yield trials were conducted each year during the time the breeding program was conducted. The new strains designated as breeder and foundation strains in Table 4 were evaluated for forage production by planting them in replicated yield trials with certain standard varieties, also given in Table 4.

It was the practice in the breeding program to form new strains from the selected plants every other year. Hence, the new strains planted in the yield trials in 1970 and 1971 were different from those planted in previous years.

Forage production of breeder and foundation seed was significantly less than that of the parent variety during the first 3 years, as shown in Table 4. However, during the last 2 years and for the average for the 5 years, the differences were not significant.

Emphasis was given to selection of plants that were free of powdery

Table 4.—Forage yields of varieties and strains of red clover on Olivier silt loam, Baton Rouge

Variety or strain	1966	1967	1968	1970	1971	Average
	Pounds hay per acre					
Tensas, parent	8,272	8,888	10,637	12,595	9,949	10,068
Nolin's	7,275	9,118	10,458	10,809	9,389	9,410
Chesapeake	—	—	8,883	9,972	9,070	9,308
Tensas, foundation	6,697	8,215	9,676	10,861	8,677	8,825
Kenland	7,924	8,417	8,999	9,940	8,645	8,785
Tensas, breeder	7,394	7,734	9,701	10,118	8,645	8,718
Orbit	7,063	7,908	8,742	10,861	8,383	8,591
LSD (5%)	1,066	627	413	NS	NS	NS

mildew during the entire time the breeding program was underway. Obviously, not all plants that showed resistance to this foliage disease excelled in forage vigor. This was recognized early in the program. Plants in the nursery showed sufficient variation for use in increasing forage production in the ensuing strains. The results from the forage yield trials show that the performance of the new strains improved in 1970 and 1971 in comparison with the standard varieties.

Forage Yields of Red Clover and White Clover

Comparisons were made of the forage yields of Tensas red clover and Louisiana S-1 white clover during the years 1964 through 1972, with the exception of 1968 when no test was conducted. The forage harvests were made in April, May, and June. Differences that were statistically significant in favor of red clover were found in the total annual yields for 5 of the 7 years the results were recorded (Table 5).

Red clover is better adapted for the production of hay than is white clover. Forage harvests were made of both species at monthly intervals and one harvest was made at full bloom. Forage production from red clover was greater when only one harvest was made at full bloom than when harvests were made at intervals. The total yield of white clover was larger when the forage was harvested at monthly intervals.

The nutritive value of red clover was found to be less than that of white clover, but was equal to that of alfalfa and certain other forage legumes.

Table 5.—Forage yields of red clover and white clover on Olivier silt loam, Baton Rouge

Year	April		May		June		Total annual yield	
	Red clover	White clover	Red clover	White clover	Red clover	White clover	Red clover	White clover
	Pounds hay per acre							
1 1964	2,245*	1,701*	1,769	1,659	2,688*	1,516*	6,702*	4,876*
2 1965	3,504	3,184	977	687	1,400*	612*	5,581*	4,483*
3 1966	3,072*	1,892*	1,804	2,198	2,377	1,674	7,253*	5,764*
4 1967	4,642	3,695	2,105	2,820	1,461	1,547	8,208	8,062
5 1969	3,235	3,305	2,545	2,314	1,187	1,060	6,967	6,679
6 1970	4,476*	4,164*	3,132	3,189	1,808*	857*	9,416*	8,210*
7 1972	5,246*	3,961*	2,800*	1,372*	929	699*	8,975*	6,032*
Average	3,774	3,129	2,162	2,034	1,693	1,391	7,629	6,301

*Differences between white clover and red clover for yield of hay in pounds per acre significant at the 5% level of probability for corresponding harvest dates.

Seed Production

Seed production by red clover has been investigated during the breeding program. It is similar to white clover in that insect pollinators are necessary for seed formation. The most effective pollinator is the bumble bee. However, with sizeable acreage and the cultural habits on most farms, it is unlikely that the natural population of bumble bees will be sufficient for effective seed set. Hives of honeybees may be supplied to serve as additional pollinators. The number of hives necessary for successful seed set will vary, but it is thought by some workers that one strong hive per acre should be adequate.

Seed production, harvesting, and cleaning are similar to that of white clover. Seed yields may vary with seasonal conditions during the growing season and the type of soil. A moderate yield of seed would be 150 to 200 pounds per acre. Yields of 400 pounds have been harvested in Louisiana.

Figure 4.—Variety yield experiment at harvest stage.

Summary

An acclimated variety of red clover was developed for use in Louisiana that was more productive than varieties developed in areas farther north.

The variety was developed from plant selections taken from seed sources found in Louisiana. The selections were tested in progeny plots; this testing was followed by new strain tests. Five outstanding lines were combined to form "Tensas," a variety name given for Tensas Parish, the place of origin of the seed from which the selections were taken.

Resistance to the disease powdery mildew, (*Erysiphe polygoni* D. C.), was established by recurrent selection for mildew-free plants made after the full-bloom stage. Blossoms and seed heads were removed from mildew-free plants, and they were forced to form seed on the second growth from the crown. The mildew-infected plants were removed from the nursery and destroyed. Selections were made from the second growth based upon vegetative vigor and seed production.

Powdery mildew infection varied in severity from year to year. It was severe during certain years, but in other years only traces could be found. This was verified by growing susceptible varieties.

Forage yields of red clover exceeded the yields of white clover significantly in 5 of the 7 years the comparative tests were conducted.

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